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Research Article

Microbiological Quality of Meat and Swabs from Contact Surface in Butcher Shops in Debre Berhan, Ethiopia

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Food-borne pathogens are the primary source of infection in developing countries. The widespread practice of raw beef consumption was a potential cause of food-borne diseases in Ethiopia. Hence, this study was initiated to assess the microbiological quality of fresh meat samples from butcher shops in Debre Berhan. Fresh meat samples and swab samples from contact surfaces were collected from butcher shops for microbial analysis, following standard methods. The study revealed that the mean microbial counts of morning samples for total aerobic mesophilic, *Staphylococci*, Enterobacteriaceae, total coliform, fecal coliform, aerobic spore formers, and yeasts and molds of the butcher shops were 5.31, 4.24, 4.47, 4.79, 4.74, 3.77, and 5.0 log cfu/g, respectively. The mean microbial counts from the afternoon sample for total aerobic mesophilic, *Staphylococci*, Enterobacteriaceae, total coliform, fecal coliform, aerobic spore formers, and yeasts and molds of the butcher shops were 5.47, 4.78, 4.84, 4.88, 4.94, 5.15, and 5.07 log cfu/g, respectively. A higher microbial load was found from the meat sample collected in the afternoon. The mean microbial counts of total aerobic mesophilic, *Staphylococci*, Enterobacteriaceae, total coliform, fecal coliform, aerobic spore formers, and yeasts and molds from swabs of the contact surface were 4.17, 3.98, 4.08, 3.96, 3.86, 3.80, and 3.92 log cfu/cm^2, respectively. Further characterization of the aerobic mesophilic flora indicated a dominance by Enterobacteriaceae (36%) followed by *Staphylococci* spp. (24%) and *Bacillus* spp. (19%). The prevalence of *S. aureus*, *E. coli*, and *Salmonella* in meat and swab samples was 37.5%, 32.5%, and 7.5%, respectively. In this study, it was observed that all samples collected from butcher shops detected a significant count of spoilage microbes. Hence, adequate sanitary measures should be taken from production to consumption stages.

1. Introduction

Foodborne diseases are usually caused by bacteria, parasites, viruses, or toxins [1, 2]. Foodborne diseases vary between countries depending on food consumption, food processing, preparation, handling, storage techniques employed, and sensitivity of the population [3]. However, the highest prevalence of foodborne outbreaks is commonly found in developing countries. Food-borne diseases associated with raw beef meat remain the most significant food safety hazards worldwide [4].

The most common genera of meat spoilage bacteria are *Staphylococcus*, *Bacillus*, *Campylobacter*, *Clostridium*, *Listeria*, *Salmonella*, lactic acid bacteria, *Pseudomonas* spp., *Acinetobacter* spp., and *Moraxella* spp. that can cause discoloration, bad odors, and slime on beef surfaces [5]. Mold species found in meat include *Cladosporium*, *Geotrichum*, *Penicillium*, and *Mucor* while yeast species include *Candida* spp. and *Cryptococcus* spp. [6]. Even though the storage conditions affect the type of microbes found in meat, slime formation, structural components degradation, biochemical change, off-odor, and appearance change were found in meat as a result of spoilage microorganisms [7].

The meat could be contaminated with microbes during slaughter and/or processing. The contaminating organisms are mainly derived from animal hide and feces. Processed meat can also be contaminated with pathogenic microorganisms from the environment during the various stages of processing [8].

In mild-to-severe illness, hospitalization or even death can be caused by the ingestion of infected food [9]. Recent data from either developing or developed countries showed that about 10% of the population may experience foodborne diseases. The situation is equally serious in developing countries, with obvious economic consequences [10]. Infected food handlers are an important source of foodborne diseases in developed countries [11].

The consumption of animal-origin food such as meats, fish, and their products especially in their raw state is generally regarded as high-risk products [12]. In Ethiopia, a habit of consuming beef at its raw or partially cooked state is common which may be a potential cause of foodborne illnesses [13]. This could be due to poor food handling and sanitation practices, inadequate food safety regulations, weak regulatory systems, and a lack of education for food handlers [14]. The meat handling and processing practices implemented in some butcher shops at Debre Berhan may provide a chance in which many spoilage microorganisms can easily grow on it and cause spoilage and food-borne disease. Therefore, the microbiological quality of meat should be assessed to devise hygienic practices implemented in butcheries to reduce food-borne disease caused by consuming spoiled products. Thus, the main objective of the study was to evaluate the microbiological quality of meat and contact surface sold in butchery shops at Debre Berhan, Ethiopia.

2. Materials and Methods

2.1. Description of the Study Area. The study was conducted in Debre Berhan which is about 130 km far from Addis Ababa, the capital city of the country. It has an elevation of 2840 meters and latitude and longitude (9041′N39032′E). Debre Berhan has a total population of 160,408 based on the 2012 national census conducted by the central statistical Agency Ethiopia [15].

2.2. Study Design. A systematic random sampling method was used to assess the microbiological quality of meat in butcher shops in Debre Berhan. For this study, fresh beef meat samples were collected from several butcher shops and intended for microbiological analysis.

2.3. Questionnaire and Observational Survey. The survey was conducted using questionnaires and visual observations. Semistructured questionnaires were prepared and filled in by food handlers and consumers in the butchers' shops to assess their knowledge, attitudes, and practices towards hygienic meat processing. A total of 16 respondents (two from each butchery shop) and 50 consumers were provided with the questionnaires to collect their responses. Level of education, exposure to training, experience of using hair cover and jewelery, the way of money handling, and personal hygiene were included in the questionnaires. A preliminary survey was conducted before the actual study to prepare and manage the relevant questionnaire.

2.4. Samples Collection. A total of 40 samples, 16 fresh beef samples (250 g each), and 24 swabs samples were collected from 8 randomly selected butcher shops, aseptically using sterile polythene plastic bags. The swab samples were taken from knives, weighing balance, and cutting tables with an area of 1 cm^2 using sterile swabs soaked into a 0.1% saline solution. Samples were then transported to Debre Berhan University microbiology laboratory using an icebox (4°C) for immediate analysis. Fresh beef samples were collected at two distinct times of the day, early in the morning (8:00-9:00 am) and late in the afternoon (5:00-6:00 pm) [16, 17].

2.5. Sample Preparation. Twenty-five grams (25 g) of the meat samples were weighed and transferred to a stomacher bag under aseptic conditions. The samples were then diluted to 10^{-1} using 225 mL peptone water and homogenized for 2 min by using a Stomacher. Following homogenization, further serial dilutions were made using sterile peptone water. On the other hand, each tube containing swab samples (10 mL of 0.1% saline water) was vortexed to ensure a mixture of the sample. A tenfold serial dilution was prepared by transferring 1 mL of the homogenized sample (both meat and swab) to 9 mL diluents. From appropriate serial dilutions, a 0.1 mL aliquot was plated on various types of media for microbial counts [16, 17].

2.5.1. Total Aerobic Mesophilic Count (TAMC). A 0.1 mL of an aliquot from appropriate dilution was pipetted and spread on standard predried plate count agar media. Inoculated plates were incubated at 32°C for 48–72 hrs. After incubation, the plates with colonies between 30 and 300 were counted using colony counter [18].

2.5.2. Total Coliforms and Fecal Coliform Count. A 0.1 mL of an aliquot from appropriate dilution was pipetted and spread on Violet Red Bile Agar. The inoculated plates were then incubated at 32°C for 18–24 hrs to determine total coliforms and at 44.5°C for 18–24 hrs to determine fecal coliform [19].

2.5.3. Enterobacteriaceae Count. To count the members of *Enterobacteriaceae*, 0.1 mL of the aliquot from appropriate dilution was spread plated on MacConkey agar (M 081 Hi-Media, Mumbai) supplemented with glucose and was incubated at 35°C for 24 hrs. All reddish purple/pink colonies were counted as members of the *Enterobacteriaceae* [19].

2.5.4. Aerobic Spore Formers. For aerobic spore-forming bacteria, the meat sample suspension was first heated at 80°C in the water bath for ten min to kill vegetative cells. Then, 0.1 mL of the homogenate was spread plated on the predried surface of plate count agar (PCA) plates. Colonies were counted after incubation at 35°C for 36 to 72 hrs.

2.5.5. Total Fungal Counts. The yeasts and molds count was done by direct plate count using Potato Dextrose Agar (M 091 Hi-Media, Mumbai) supplemented with 0.1 g of

chloramphenicol as an antibacterial agent. A 0.1 mL from the appropriate dilution was spread plated on PDA. Total yeasts and molds were counted after incubation at 25°C for 3–5 days [20].

2.6. Detection of Pathogenic Microorganisms

2.6.1. Total Staphylococcus spp. Count. Staphylococcus species were enumerated by pour plate method and grown on Mannitol Salt Agar (MSA). 0.1 mL aliquot from the appropriate dilution was inoculated into predried MSA plates. The inoculated plates were incubated at 37°C for 24 hrs. After incubation, yellow colonies were counted and recorded as *Staphylococcus* counts using the colony counter [19].

2.6.2. Detection of Escherichia coli. E. coli species were isolated using MacConkey agar (Hi-Media, Mumbai, India). 0.1 mL of the sample was spread into MacConkey agar plates and incubated at 37°C for 24 hrs. The colonies were confirmed by streaking 2-3 colonies onto MacConkey agar and colonies were further confirmed by Gram's staining and by biochemical tests [21, 22].

2.6.3. Detection of Salmonella spp. A 25 g of meat sample (minced by stomacher) was transferred to 225 mL of Buffered Peptone Water (BPW) and incubated at 37°C for 24 hrs. An aliquot of 0.1 mL from perenrichment was pipetted to 10 mL tetrathionate broth (supplement with iodine). A loopful sample from tetrathionate culture was streaked onto SS agar plates. The plates were incubated at 37°C for 24 hrs. After 24 hrs of incubation, the formation of colonies with black centers or with gray colors on SS agar was considered as presumptive *Salmonella* spp. [23, 24].

2.7. Determination of Microbial Load. Appropriate plates containing distinct microbial colonies were selected and counted using a colony counter. Then, the microbial load was determined using the standard formula as follows [25]:

$$N = \frac{n}{s \times d}, \quad (1)$$

where N = total number of bacteria (cfu) per gram of the sample, n = average number of bacterial colonies, from different dilutions in Petri dish that contained 30–300 colonies, s = volume of sample for plating, and d = dilution factor of the specimen/food sample.

2.8. Characterization of Dominant Microorganism. After enumeration, from plate count agar (total aerobic mesophilic bacteria), about 5 colonies were picked randomly from countable plates and inoculated into tubes containing about 5 mL nutrient broth. These were then incubated at 30°C overnight. The cultures were purified by repeated streak plating and characterized using morphological and biochemical tests [26].

2.8.1. Morphological Characterization of Dominant Bacteria

(1) Cell Shape and Cell Arrangement. From overnight pure broth culture, the wet mount was prepared on a microscope slide and stained using methylene blue. The stained microbial cells were then observed under a light microscope using an oil immersion objective (x100). The morphological criteria considered during the observation were cell shape (spherical, rod, spiral, etc.) and cell arrangement (single, pair, chain, clusters, and tetrads) [27].

(2) Gram Reaction (KOH Test). The KOH test was conducted according to the method of Gregerson [28]. Twenty-four-hour-old pure culture colony picked from the plate count agar was placed on a clean slide and stirred with two drops of 3% KOH for about 2 min. The Gram-negative mass was allowed to rise with inoculating needle following the loop to raise 0.5 to 2 cm or more whereas the Gram-positives did not show slime.

2.8.2. Biochemical Tests

(1) Catalase Test. Young colonies are flooded with a 3% solution of hydrogen peroxide. The formation of bubbles was considered as the presence of catalase [26].

2.9. Biochemical Tests for Escherichia coli sp. The presence of *E. coli* spp. was confirmed using indole and MRVP test [22, 26].

2.10. Detection of Staphylococcus aureus. For identification of *S. aureus*, a loopful of the sample from the homogenate was inoculated onto Mannitol salt agar. Then, golden-yellow colonies on MSA which showed catalase-positive and coagulase-positive were considered as *S. aureus*. The biochemical tests (catalase and coagulase) were done as a confirmatory test [19].

2.11. Biochemical Tests for Salmonella spp. The biochemical test for *Salmonella* spp. was conducted using Triple Sugar Iron Agar test [22, 26], Lysine Iron Agar test ([24] and ISO, 6579), and Simmons Citrate Agar test.

2.12. Data Analysis. The data analyses were performed using Statistical Package for Social Sciences (SPSS) version 20. One-way ANOVA and LSD were performed for mean comparison at $P \leq 0.05$ using the same program [29].

3. Results

3.1. Observation and Questionnaire Survey. The data were collected from 16 meat handlers (13 men and 3 women) working at 8 butcher shops using questioners and visual observations (Tables 1 and 2). Out of the total participants, 5 were illiterate while the remaining 11 completed primary school (Table 1). Regarding the training experience, only 4 (25%) out of 16 participants had taken training on sanitary practices. Only 5 out of 16 participants had renewed their

Table 1: Survey on knowledge of butchers on hygienic practices in Debre Berhan town.

Variables	Value	Frequency	Percent
Sex	Male	13	81
	Female	3	19
Educational level	Illiterate	3	19
	Primary school	11	68
	High school	2	13
Experience of training	Yes	4	25
	No	12	75
Health certificate	Yes	5	31
	No	11	69
Clean overcoat	Yes	12	75
	No	4	25
Hair cover	Yes	9	56
	No	7	44
Wearing watch and jeweleries	Yes	6	37
	No	10	63
Skin rash, skin boils, cut, diarrhea, and vomiting	Yes	3	19
	No	13	81

health certificates. Concerning their hygiene, 75% of the meat handlers wore clean overcoats and while 56% used hair covers (Table 1). On the other hand, only 19% of the food handlers had a skin rash, skin boils, and/or skin cut (Table 1).

A total of 50 respondents participated in this study by providing their opinion and experiences related to sanitary practices used in the butcher's shop. Out of the total respondents, 41 (82%) and 9 (18%) were male and female, respectively. Twelve (24%) respondents completed secondary school whereas 19 (38%) respondents completed college and above. Most 38 (76%) of the respondents prefer beef to other types of red meat and 44 (88%) of them preferred fresh meat for consumption. Only 4 (8%) of the respondents considered the healthiness of the meat (Table 3). 42 (84%) of the respondents showed the habit of raw meat consumption. More than half (58%) of the respondents did not recognize fecal-oral pathogen transmission. About 44% of the respondents suffered from food poisoning, of which 17 (34%) required medical attention and took antibiotics for recovery (Table 3).

3.2. Microbial Dynamics in Meat and Contact Surface for Samples Collected from Butcher Shops

3.2.1. The Microbial Load of Meat. The mean total aerobic mesophilic bacteria (TAMC), aerobic spore formers (ASFC), *Enterobacteriaceae* (EBC), total coliform (TCC), fecal coliform (FCC), total St*aphylococci* (TSC), and yeast and mold (YMC) in the meat collected during the morning and afternoon periods were found between 5.31 and 5.47 log cfu/g, 3.77 and 5.15 log cfu/g, 4.47 and 4.84 log cfu/g, 4.79 and 4.88 log cfu/g, 4.74 and 4.94 log cfu/g, 4.24 and 4.78 log cfu/g, and 5.0 and 5.07 log cfu/g, respectively (Table 4). In all the cases, higher microbial counts were detected from beef meat samples collected in the afternoon as compared to the morning.

3.2.2. The Microbial Load of Swabs from the Contact Surface of Butcher Shops. Mean total aerobic mesophilic bacteria (4.17–4.84 log cfu/cm^2), aerobic spore formers (3.80–4.74 log cfu/cm^2), *Enterobacteriaceae* (4.08–4.26 log cfu/cm^2), total coliform (3.67–4.41 log cfu/cm2), fecal coliform (3.86–3.94 log cfu/cm^2), total *Staphylococci* (3.98–4.81 log cfu/g), and yeast and mold (3.61–4.53 log cfu/cm^2) were detected from contact surfaces (knife, cutting table, and balance) (Table 5). The highest count for all groups of microorganisms was noticed from knife swabs except for *Enterobacteriaceae* and fecal coliform. On the other hand, the maximum counts for *Enterobacteriaceae* and coliform were detected from the cutting table and balance, respectively.

3.2.3. Prevalence of Pathogenic Microorganisms and Distribution. A food-borne illness caused by nontyphoid *Salmonella*, *S. aureus*, and *E. coli* represents a major public health problem worldwide. These pathogens are transmitted mainly through the consumption of contaminated food and the presence of these organisms in raw meat products have relevant public health implications. In this study pathogens like *Salmonella*, *S. aureus*, and *E. coli* were identified using different biochemical tests such as catalase, oxidase, triple sugar iron, Simmons citrate, lysine iron agar, motility, indole, methyl red, gas production, H_2S production, acid production, coagulase, and gram reaction test (Table 6).

A total of 40 samples (16 beef meat and 24 swabs from contact surfaces) were analyzed to check the presence of pathogenic microorganisms. According to the biochemical test result in Figure 1, 15 (37.5%), 13 (32.5%), and 3 (7.5%) samples were presumptively detected as positive for *S. aureus*, *E. coli*, and *Salmonella* spp., respectively. In all the cases, the maximum positive samples were detected from beef meat in comparison to samples from contact surfaces.

Table 2: Hygienic data collected from the butcher shops by visual observation.

Variables	Value	Frequency	Percent
Means of money collection	Cashier	5	63
	Butchers	3	37
Refrigerator usage	Yes	0	0
	No	8	100
General sanitation of the shop	Better	2	20
	Good	1	17
	Poor	5	63
Location of the toilet and possibility of contamination	Yes	2	20
	No	6	80
Availability of soap for washing hands	Yes	0	0
	No	8	100

Table 3: Consumers' knowledge, attitudes, and practices regarding meat preference and foodborne diseases.

Variables	Value	Frequency	Percent
Sex	Male	41	82
	Female	9	18
Education status	Illiterate	11	22
	Primary school	8	16
	High school	12	24
	College and above	19	38
Age	15–30	13	26
	31–50	21	42
	Above 50	16	32
Priority criteria	Freshness	44	88
	Healthiness	4	8
	Cheapness	2	4
Red meat you prefer	Beef	38	76
	Mutton	12	24
Do you consume raw beef	Yes	42	84
	No	8	16
How often do you consume	Every day	0	0
	Once in week	17	34
	1–3 times a week	1	2
	Once per month	32	64
History of food infection	Yes	22	44
	No	28	56
Symptoms shown	Vomiting	6	12
	Abdominal cramp	27	54
	Nonbloody diarrhea	17	34
Types of action taken	Drug	17	34
	Traditional	21	42
	None	12	24
Pathogen transmitted by meat consumption	Yes	21	42
	No	29	58

3.2.4. Dominant Microflora. A total of 88 bacterial isolates were isolated and categorized into different bacterial genera using different biochemical tests such as oxidase, catalase, motility, indole, and gram reaction (Table 7). Consequently, the aerobic mesophilic flora was dominated by Enterobacteriaceae (36%) followed by *Staphylococci* spp. (24%) and *Bacillus* (19%). *Streptococci* (10%) and *Pseudomonas* (7%) were found among the aerobic mesophilic bacteria isolated from beef and contact surface samples (Figure 2).

4. Discussion

The educational level of all the meat handlers included in the survey of the present study was below secondary school (Table 1). Most meat handlers (75%) who participated in the study had a lack of training on hygienic meat processing practices. This could be due to low-scale knowledge of the meat handlers regarding food handling and processing practices [9]. The safety of food can be insured by providing

Table 4: Microbial count in fresh beef meat from butcher shops (collected during morning and afternoon time) expressed in log 10 cfu/g ± standard deviation.

Bacteria	Fresh meat samples collected from butcher shops at different time	
	Morning time Log cfu/g mean ± SD	Afternoon time Log cfu/g mean ± SD
Total aerobic bacteria	5.31 ± 1.27^{b}	5.47 ± 1.40^{a}
Aerobic spore formers	3.77 ± 0.03^{b}	5.15 ± 0.26^{a}
Total coliform	4.79 ± 0.61^{b}	4.88 ± 1.29^{a}
Enterobacteriaceae	4.47 ± 0.55^{b}	4.84 ± 0.92^{a}
Total fecal coliform	4.74 ± 0.91^{b}	4.94 ± 0.75^{a}
Total *Staphylococci*	4.24 ± 0.16^{b}	4.78 ± 0.61^{a}
Yeasts and molds	5.00 ± 0.41^{a}	5.07 ± 0.44^{a}

CFU/g: colony-forming unit per gram of beef meat; SD: standard deviation; mean: average of three measurements. Different letters (a, b) in the same rows have significantly different means as determined by JMP Pro 13 a SAS comparison test ($P < 0.05$).

Table 5: Microbial count from contact surface (knife, cutting table, and balance) expressed in log cfu/cm^2 ± standard deviation.

Bacteria	Log cfu/cm^2 counts from contact surfaces								
	Knife ($n = 8$)			Cutting table ($n = 8$)			Balance ($n = 8$)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
TAMC	4.84^{a}	4.30	6.50	4.17^{c}	3.67	4.73	4.32^{b}	3.82	4.73
ASFC	4.74^{a}	4.51	5.23	3.80^{c}	3.61	3.96	3.93^{b}	3.61	4.45
EBC	4.23^{b}	3.54	5.00	4.26^{ab}	3.65	5.70	4.08^{c}	3.56	4.97
TCC	4.41^{a}	3.69	5.56	4.23^{b}	3.79	4.89	3.96^{c}	3.67	4.56
FCC	3.93a	3.51	4.53	3.86^{b}	3.54	4.40	3.94^{a}	3.7	4.54
TSC	4.81^{a}	4.38	6.69	4.16^{b}	3.66	4.66	3.98^{c}	3.59	4.69
YMC	4.52^{a}	3.61	5.58	3.99^{b}	3.67	4.45	3.92^{c}	3.61	4.54

Means in the same row with different superscript letters (a, b, c, and ab) are significantly different means as determined by JMP Pro 13 a SAS comparison test ($P < 0.05$).

Table 6: Biochemical test for pathogenic microorganisms.

Biochemical test	*E. coil*	*S. aureus*	*Salmonella*
Catalase	+ve	+ve	+ve
Coagulase	−ve	+ve	−ve
Oxidase	−ve	−ve	−ve
Triple sugar iron gas, H_2S, and acid production	Gas and acid produced	Acid production	H_2S production
Simmons citrate	−ve	+ve	−ve
Lysine iron agar	H_2S production		H_2S production
Indole	+ve	−ve	−ve
MRPV	+ve MR and −ve VP	+ve	+ve MR and −ve VP
Gram reaction	−ve	+ve	−ve

−ve = negative result and +v = positive result.

training to food handlers concerning basic concepts and personal hygiene [30], and 58.33% of butcher shop workers in Bishoftu, Ethiopia, did not receive training regarding meat handling practice [30]. Different from the present study, about 60% of the meat handlers in Mekelle had taken training concerned with personal hygiene and food handling practices (Balcha and Gebretinsae [16]).

In this study, only 19% (3) of the food handlers had skin lesions. In the same way, food handlers who suffer from jaundice, diarrhea, vomiting, fever, sore throat with fever, discharge from the ear, or eye or nose or have visibly infected skin lesions were not allowed to work as food handlers according to the guidelines of WHO [31].

The personal hygiene of meat handlers showed that 75% wore clean working coats while 56% used hair covers during serving their customers (Table 1). Thus, the current finding fits with WHO regulation that states, food handlers must dress in clean and suitable clothing [31]. Similarly, Balcha and Gebretinsae [16] also reported that about 58% and 42% of the food handlers working at butcher shops in Mekelle wore overalls and hair covers, respectively.

In the present study, the general sanitation statuses of the butcher shops were poor. Similarly, Zerabruk et al. [32] reported the hygienic conditions of most of the butcher shops involved in the study were poor and the meat products were not separated from offal. Ali et al. [33] also reported

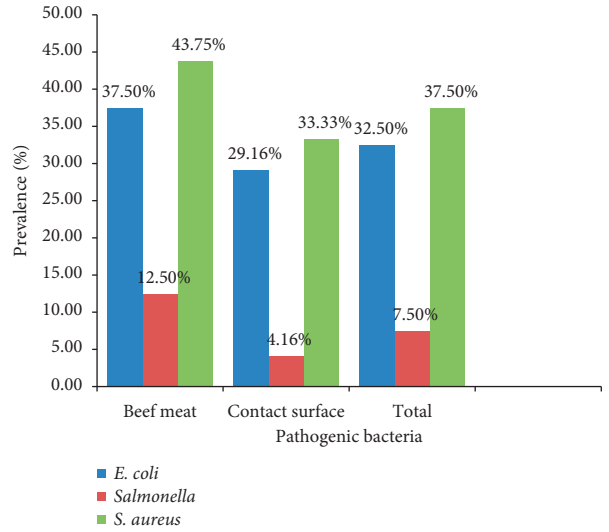

Figure 1: Prevalence of pathogenic bacteria in beef meat and swabs from contact surfaces.

Table 7: Biochemical test for total aerobic mesophilic bacteria.

Isolates from PCA	Biochemical test					Gram reaction and morphology
	Oxidase	Catalase	Motility	Indole	Bacteria genus	
1	−ve	+ve	+ve	−ve	*Enterobacteriaceae*	Gram-negative and rod
2	+ve	+ve	+ve	−ve	*Staphylococci*	Gram-positive and cocci
3	−ve	+ve	+ve	−ve	*Bacillus*	Gram-positive and rod
4	+ve	−ve	+ve	−ve	*Streptococci*	Gram-positive and cocci
5	+ve	+ve	+ve	−ve	*Pseudomonas* spp.	Gram-negative rod
6	+ve	+ve	−ve	−ve	*Micrococcus*	Gram-positive and cocci

−ve = negative and +ve = positive.

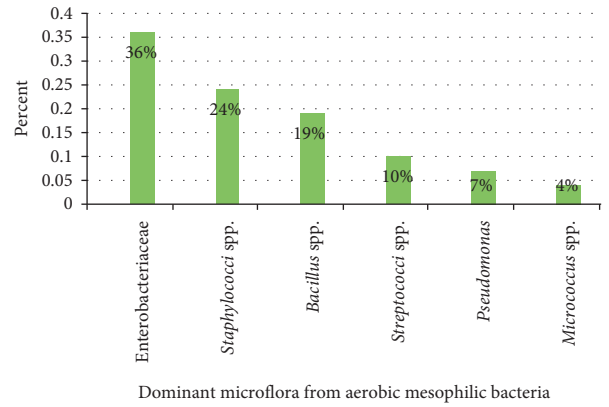

Figure 2: Dominant bacterial genera from aerobic mesophilic flora.

that most of the surveyed butcher shops had poor hygienic conditions concerning the cleaning of their shops and lack of knowledge about disinfection. Poor handling and hygienic practices led to high cross-contamination and recontamination of meat [34]. Most of the meat products in the butcher shops were held on hangers or tables for more than 11 hours. This practice may give sufficient time for the growth of spoilage/pathogenic microorganisms. Ali et al. [33] reported that cleaning butcher shops with detergent and water once in 24 hours is not enough to maintain hygienic environments in the butcher. In agreement with this study, Muleta and Ashenafi [35] reported that microbes duplicate dramatically if food stays at temperatures between 15 and 45°C for greater than 4 hrs.

In this study, about 63% of the butcher shops have cashiers for money collection. Hence, it reduces the possible

contamination of meat by spoiling microorganisms, which could cause a major health hazard [36]. Differently, Balcha and Gebretinsae [16] reported that 92% of the food handlers in Mekelle butcheries handled money and serving food, simultaneously.

About 76% of the consumers included in the study preferred to eat beef meat and 84% preferred to eat raw meat. Out of the total respondents that preferred meat in its raw state, 84% of them consumed raw beef which may perhaps be the potential source of spoilage/pathogenic microorganisms. Sisay [37] reported that 55 (91.7%) of the consumers prefer beef to other types of meat, and 42 (70%) prefer eating raw to other types of preparations in Dukem town. Contaminated raw meat is one of the main sources of food-borne illness [38, 39].

In this study, significant microbial counts were detected in all beef meat samples for all groups of microorganisms. However, the highest microbial counts were recorded from beef meat samples collected in the afternoon time (Table 4). Hence, the microbial load from afternoon samples increased by 0.07 log cfu/g for yeast and molds and 1.38 log cfu/g for aerobic spore formers as compared to the morning samples. The higher microbial count recorded in the afternoon meat samples could be due to an open display of meat for a prolonged period that may favor suitable conditions for microbial growth. A study done in Ghana on the TPC from beef in open markets found the mean TPC to be 6.36–8.47 log cfu/g [40], and Ali et al. [33] reported a higher mean TPC on beef samples from the open market. Similarly, a study conducted in Northern Ghana has reported 2 log differences between morning and afternoon in microbial count from meat samples [17]. Another study conducted in Ghana supermarkets found the mean total plate counts to be 5.01–8.32 log cfu/g [40].

The mean TAM count (5.31 log cfu/g, morning, and 5.47 log cfu/g, afternoon) detected from the fresh beef meat in this study was lower than the study conducted by Zerabruk et al. [32] on minced meat from Addis Ababa. However, the average ASF count (3.77–5.15 log CFU/g) recorded in this study was higher than the mean ASF (2.35–2.42 log cfu/g) count reported by Zerabruk et al. [32]. The mean TSFC (4.24–4.78 logs/g) recorded in the present study was lower than TSFC (5.8–7.5 log CFU/g) reported by Tafese [41]. Similarly, a mean count of TAM (±5.57 cfu/g) from Ghana [42] and a mean count of TAM (7.15 cfu/g) [43] were reported. TAM count obtained from the butcheries during the study exceeded the accepted range (>5.0 cfu/g) and hence no meat sampled from the butcheries during the study was fit for consumption [44, 45].

The mean EC count (4.47–4.84 log cfu/g) and CC count (4.74–4.94 log CFU/g) noticed from meat samples in the current study was comparative to the finding reported by Tafese [41] whereas they were lower than the EC and CC count described by Zerabruk et al. [32]. In general, substantial EC, TC, and FC counts recorded in this study indicate the possible spoilage of the product [46]. According to EC [47] and FSSA [48], the acceptable standards for CC and EBC are 4 cfu\g and 3 cfu\g respectively. The highest fecal count recorded in the present study might be due to cross-contamination from the gut of the animals and/or direct fecal contamination [49].

The significant *Staphylococci* count (4.24 log cfu/g in the morning and 4.78 log cfu/g in the afternoon) detected in the present study could be due to inappropriate individual hygiene of food handlers and cross-contamination from skin and utilities under poor sanitary conditions (Table 4). The total *Staphylococci* count determined from minced meat (4.57 log cfu/g in the morning and 5.77 logs cfu/g in the afternoon) at Addis Ababa city was comparative with the present study [32]. Contrarily, the *Staphylococci* count determined from Arsi cattle meat at Adama (4.98–6.01 log cfu/-g) was higher than this study [50]. The variation may be acquired due to differences in ambient temperature between the study area (Debre Berhan and Adama). The yeast and mold count recorded from beef meat (5.00–5.05 log cfu/g) in this study was lower than the yeast and mold count (5.59–6.04 log cfu/g) noticed from minced meat in Addis Ababa [32].

The mean TAMC noticed from contact surfaces in the present study was slightly lower than the finding reported for the same microorganisms from the knife (6.31 log cfu/cm^2), cutting table (6.32 log cfu/cm^2), and balance (6.43 log cfu/cm^2) [32]. Similarly, Balcha and Gebretinsae [16] reported a higher TAMC of 6.56 log cfu/cm^2 and 6.78 log cfu/cm^2 from the table and knife, respectively. The high TAM count detected from contact surfaces indicated insufficient sanitary conditions in the butcheries.

The mean total *Staphylococci* count noticed from the knife, cutting table, and weighing balance were 4.81, 4.16, and 3.98 log cfu/cm^2, respectively. The presence of *Staphylococci* in all the samples in such density indicated unacceptable hygienic standards particularly poor personal hygiene. The *Staphylococci* count recorded from meat contact in the UK was lower than the current study [51]. The differences in mean count between the two countries may indicate variation in personal hygiene practices. *Staphylococcus* spp. can be part of normal flora on the skin of humans and animals which can be transmitted from person to product through unhygienic practices [52]. Gracey and Collins [53] also noticed that the meat product with high contact with human hands is associated with reasonable changes of *Staphylococci* spp.

In this study, the mean total coliform from the knife, working table, and balance was 4.41, 4.23, and 3.96 log cfu/cm^2, respectively, which was higher than the comparative study in the UK [51]. The TCC noticed from knife and table in the present study was lower than the comparative study (TC of knives 5.51 log cfu/cm^2 and TC of working tables 5.34 log cfu/cm^2) conducted in Addis Ababa [32]. Fecal coliform count from cutting board and knife (5.80 and 5.83 log cfu/cm^2) of the study conducted by Ayalew et al. [54] in Jijiga city was higher than the present study. The occurrence of cross-contamination and immediate contamination may increase the count.

The mean yeast and mold count recorded from the contact surface (3.92–4.52 log cfu/cm^2) in this study was lower than the study conducted in Jijiga [54]. In general, the high microbial load found on the contact surface may

indicate the presence of significant cross-contamination among the contact surfaces during the operation.

Among all the 40 samples tested for the presence of pathogenic microbes, 15 (37.5%) were positive for *S. aureus*. The high contamination of food with *S. aureus* has been related to inappropriate personal hygiene of the food handlers during handling and processing of meat products [55] and indicates the health hazards of consuming raw meat handled under unsanitary circumstances [25].

Similar to the present study, about 31% [25] and 29.4% [54] of the meat samples were positive for coagulase-positive *S. aureus* and *Staphylococcus* spp. respectively. However, only 24.53% of the meat samples were positive for *S. aureus* [55]. In another study, a higher prevalence rate of *S. aureus* than this study was detected from the contact surface materials in butcher shops [16].

A higher prevalence of *E. coli* (32.5%) was also detected from meat and contact surfaces of materials. Hence, the significant positive samples for *E. coli* noticed in this study showed the unhygienic meat handling practices in butcher shops. Even though the presence of *E. coli* in foods is not always alarming because most strains are harmless and opportunistic, the presence of harmful strains (*E. coli* O157) can pose gastroenteritis by producing Shiga toxin [58]. Similar to the present study, about 30% [59] and 32% [16] were positive for *E. coli* in meat and contacts surface samples collected from butcher shops.

E. coli, *Staphylococcus aureus*, *Salmonella* sp., and *Proteus* sp. were prevalent in beef samples [60]. In the present study, a few numbers of samples collected from butcher shops showed the presence of presumptive *Salmonella* spp. with a prevalence rate of 7.5%. This finding was supported by [61] that reported in Addis Ababa, about 12%, *Salmonella* in raw retail meat in Addis Ababa, and in the other study, 3% of sheep carcasses were positive for *Salmonella* [62]. The contamination of meat with *Salmonella* at retail is due to unhygienic carcass transportation, improper loading and unloading, unhygienic meat shop equipment, and personnel [61]. However, the detection of *Salmonella* in any sample could be due to poor hygiene and sanitary practices through all value chains of the meat supply and indicated the potential risk associated with the consumption of these foods [63]. *Salmonella* infections continue to be a major public health concern in many countries and the infections are generally foodborne [64].

The microbial flora recovered from beef meat and contact surface were dominated by Enterobacteriaceae, *Staphylococci*, and *Bacillus* spp. Likewise the microflora of "kitfo" was dominated by the same spp. [35]. In another study, Dabessa [65] also reported the dominance of *Bacillus*, *Staphylococcus*, and Enterobacteriaceae spp. in household meat in Jimma. Similar findings reported that bacteria isolates from minced meat were dominated by *E. coli*, coliform, *S. aureus*, *Streptococcus* spp., and nonlactose fermenter bacteria [66]. Bacteria isolated from butcher shops' hanged meat and minced meat were Enterobacteriaceae, *Staphylococcus*, and *Streptococcus* species [67].

Bacterial identification was conducted by standard biochemical methods [68]. For Gram-negative organisms, further identification tests included oxidase, citrate, triple sugar iron, and catalase were done [69]. Discrete colonies were subcultured into fresh agar plates aseptically to obtain pure cultures of the isolates and used for further identification using biochemical methods [70]. Bacterial identification was conducted by standard biochemical methods (Manual of Clinical Microbiology, 2002). A biochemical tests were done for Gram-negative organisms, by oxidase, citrate, triple sugar iron, and catalase according to Bauer et al. [59, 67, 68].

5. Conclusion

The major factors that contributed to the contamination of meat were low-level awareness of hygienic practices, unproper handling of paper currency, and poor sanitation of the butcher shops. In this study, a significant microbial load of spoilage microbes was noticed in beef meat and swabs from contact surfaces. However, the highest microbial loads were detected from the meat samples collected in the afternoon time. Significant samples were positive for pathogenic microbes that may lead to the risk associated with the consumption of the products.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding this paper.

Authors' Contributions

Tefera Atlabachew was involved in acquisition and analysis, interpretation of data, and drafting of the work. Jermen Mamo participated in conception, acquisition, analysis, interpretation of data, drafting of the work, analysis, and substantive revision of the work. The authors agreed to publish this research article.

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